

APPENDIX

IN THE SPECIFICATION:

Please amend the table on page 13 as follows:

Table 1 – Amino acid substitutions in the KNT mutant strains

SEQ ID NO:	Residue No.	2	17	25	57	61	62	66	75	91	94	102	112	116	117	159	188	190	196	197	198	199	203	206	207	211	220	234	238	246
1	WT*	N	H	D	M	E	A	H	V	Q	S	Q	S	L	E	T	S	S	V	K	Q	S	S	D	H	F	S	L	T	D
12	KT3-1	Y							A	R		R	P	F					L									V	A	
13	KT3-3		N	L	G	V			A	P	R			G					L							L				
14	KT3-5				G		Y	A	R	P	R											P						V		N
15	KT3-7	S			G		Y	A	R		R	P	F						T		L							V		
2	KT3-11	K			G		Y	A	R		R	P	F									P				L				
16	KT3-12					T	Y	A	R	P	K	T			L	G				R		P	P						A	
17	KT3-13				G		Y	A	R		R	P	F									P		V	Q					
18	KT-3-15				G		Y	A	R		R	P	F									P		V	Q					
19	KT3-16				G		Y	A	R	P	K	P										P		V	Q		P			
20	KT3-19			L			Y	A	R	P	K	P										P		V	Q					
3	HTK	K		L	G	V	Y	A	R	P	R	P	F									P	P	V	Q	L	P	V	A	

IN THE CLAIMS:

1. A mutant kanamycin nucleotidyltransferase [having] comprising the sequence of SEQ ID NO:1 modified by at least one [or more] point mutation [mutations] selected from [a group consisting of] Met57Leu, [Ala62Val,] Ser94Pro, Ser203Pro, Asp206Val, His207Gln, Ser220Pro, Ile234Val and Thr238Ala [as against the protein comprising the amino acid sequence indicated by SEQ ID NO: 1], and having improved thermostability as compared to SEQ ID NO:1.

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2. A mutant kanamycin nucleotidyltransferase with improved thermostability
[, wherein it comprises] as compared to SEQ ID NO:1, comprising the amino acid
sequence indicated by SEQ ID NO:2. [SEQ ID NO: 2.]

3. The mutant kanamycin nucleotidyltransferase according to claim 1,
comprising [wherein it comprises] the amino acid sequence indicated [in SEQ ID NO:
3.] by SEQ ID NO:3.

IN THE ABSTRACT:

It is desirable to have [To obtain a] selective markers [marker] suitable for
screening of thermophilic bacteria such as *Thermus thermophilus*. *T. thermophilus* are
good research materials for investigating the interrelation between enzyme structures
and functions since they are stable at extreme pH, crystallize easily and are easy-to-
handle.

[To provide a novel] Novel mutants of *Staphylococcus aureus* kanamycin
nucleotidyltransferase with markedly improved thermostability are disclosed, as well as
a selective marker using the same, and a screening method for thermophilic bacteria
such as [*Thermus*] *T. thermophilus* using said selective marker.

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12	KT3-1			Y					A	R		R	P	F																		
13	KT3-3				N	L	G	V			P	R		G				L								L						
14	KT3-5						G		Y	A	R	P	R																			N
15	KT3-7	S					G		Y	A	R	R	P	F				T			L		P									
2	KT3-11	K					G		Y	A	R	R	P	F								P	P	P		L						
16	KT3-12						T	Y	A	R	P	K	T	P	F		L	G		R		P	P									A
17	KT3-13						G	Y	A	R	R	R	P	P	F							P	P	V	Q							
18	KT-3-15						G	Y	A	R	R	R	P	P	F							P	P	V	Q							
19	KT3-16						G	Y	A	R	P	K	P	P								P	P	V	Q			P				
20	KT3-19				L			Y	A	R	P	K	P	P								P	P	V	Q							
3	HTK	K			L	G	V	Y	A	R	P	R	P	F								P	P	V	Q	L	P	V	A			

KT3-15 has the same mis-sense mutation as KT3-13. These two mutants, share three silent mutations, however KT3-13's two silent mutations and KT3-15's one mutation are mutually specific to each. Therefore, it is clear that these two mutants are distinct clones.